Abnormal Prenatal Cell-Free DNA Screening Results

What do they mean?

What is cell-free DNA screening (cfDNA)?

cfDNA screening (also referred to as non-invasive prenatal testing, NIPT, or non-invasive prenatal screening, NIPS) is a screening test that utilizes bioinformatic algorithms and next generation sequencing of fragments of DNA in maternal serum to determine the probability of certain chromosome conditions in a pregnancy. All individuals have their own cell-free DNA in their blood stream. During pregnancy, cell-free DNA from the placenta (predominantly trophoblast cells) also enters the maternal blood stream and mixes with maternal cell-free DNA. The DNA of the trophoblast cells usually reflects the chromosomal make-up of the fetus.

cfDNA routinely screens for trisomy 21, trisomy 18 and trisomy 13. Screening for fetal sex, sex chromosome aneuploidy, other aneuploidies, triploidy, and specific microdeletion conditions is also available. Conditions included on the cfDNA panel vary based upon the performing laboratory. cfDNA cannot screen for all chromosome or genetic conditions.

What does an abnormal cfDNA result mean?

Abnormal results indicate an increased risk for the specified condition. However, an abnormal result is not diagnostic and patients should be offered confirmatory testing through a diagnostic procedure, such as amniocentesis. An abnormal result may indicate an affected fetus, but can also represent a false positive result in an unaffected pregnancy, confined placental mosaicism, placental and fetal mosaicism, a vanishing twin, an unrecognized maternal condition or other unknown biological occurrence.

What is the difference between a "Positive" or "Aneuploidy Detected" result and a ">99%" risk score?

The difference in reporting is laboratory specific, however all mean the same thing: there is an increased risk. These results represent findings in the cfDNA and may not represent the chromosomal make-up of the fetus. A >99% risk score does not mean there is a greater than 99% chance that the pregnancy is affected with a condition. A "Positive" or "Aneuploidy Detected" does not mean the fetus definitively has a chromosome condition.

How accurate is an abnormal result?

These tests are often advertised to patients and healthcare providers as being >99% accurate. It is important to recognize that this is a population-level statistic and applies to all women screened. Since most pregnancies are unaffected and most results are "low risk" this test is correct 99% of the time for all women. However, the chance that a high risk result indicates an affected fetus is not 99% in the majority of cases. In order to determine the chance for a high risk result to be a true positive, one must calculate the positive predictive value.

What is positive predictive value?

Positive predictive value (PPV) is the proportion of positive results that are true positives. In other words, PPV answers the question: "What is the chance an abnormal cfDNA result means the fetus has this condition?" PPV is dependent not only on the sensitivity and specificity of the test, but it is highly dependent on the prevalence of the condition. Data from five different studies evaluating PPV of cfDNA screening are summarized below.

Positive Predictive Value	Wang et al.	Bianchi et al.	Choy et al.	Meck et al.	Norton et al.
Trisomy 21	38/41 (93%)	5/11 (45.5%)	52/55 (95%)	29/30 (97%)	9/47 (80.1%)
Trisomy 18	16/25 (64%)	2/5 (40%)	6/12 (50%)	3/5 (60%)	9/10 (90%)
Trisomy 13	7/16 (44%)		4/7 (57%)	1/4 (25%)	2/4 (50%)
Sex Chromosome Aneuploidy	6/16 (38%)		4/6 (67%)	1/7 (14%)	

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As demonstrated by these studies, PPV varies by condition, the study population and incidence of a condition in that population (the a priori risk), as well as the sensitivity and specificity of the cfDNA screen. While studies support that cfDNA screening has a higher PPV than traditional screening tests it is important to note that these PPVs cannot be universally applied to patients. The PPV will be higher for patients who have a higher a priori probability based on age or other screening results; the PPV will be lower in women with a lower a priori risk. For instance, with all else equal, the PPV is higher for women at age 40 than it is at age 20 because the a priori risk of aneuploidy increases with maternal age.

How do I explain these results to my patient?

You can explain these results similarly to how you would explain other screening tests, taking into consideration cfDNA screening has fewer false positive results than traditional screening. While an abnormal result greatly increases concern, it does not provide a diagnostic answer and further testing is necessary for confirmation. The degree of concern is highly dependent on the sensitivity and specificity of the screen and the prevalence of the disorder, which may be affected by the specific condition, maternal age, gestational age, ultrasound findings and family history.

For example, a 44 year old who has an increased fetal nuchal translucency measurement and an abnormal cfDNA result for Down syndrome, has a very high chance of having a true positive result (because the prevalence and therefore PPV is high in this population). Compare this to a 24 year old with a normal ultrasound and an abnormal cfDNA result for trisomy 13, who has a lower chance of having a true positive (because the prevalence and therefore PPV is low in this population). Like traditional maternal serum screening, not all abnormal results indicate the same degree of risk.

What if there are also abnormal ultrasound findings?

Although abnormal ultrasound findings may increase suspicion for a particular condition, ultrasound is not diagnostic and should be used as another tool to assess risk. Invasive testing is needed to definitively confirm the diagnosis and assist with genetic counseling for recurrence risk assessment, prognosis, and management.

What are the next steps?

Confirmatory testing via CVS or amniocentesis should be offered to all women with an abnormal cfDNA result. Patients electing to have CVS for confirmation should be counseled regarding the limitations of this technology since confined placental mosaicism may explain the abnormal cfDNA and may be detected with CVS, especially when aneuploidy FISH is ordered. Trophoblast cells are the primary source of cell free DNA in maternal blood and are also the cells analyzed for aneuploidy FISH testing. Confined placental mosaicism may cause an abnormal cfDNA result and abnormal CVS FISH and/or karyotype result. Cells analyzed by amniocentesis are not typically affected by confined placental mosaicism since they are primarily derived from the fetal skin and genitourinary tract.

What if my patient declines invasive testing?

If diagnostic testing is declined, management of the pregnancy should be dictated by ultrasound findings and maternal indications. Additional ultrasounds and fetal echocardiogram screening may be considered when cfDNA results are abnormal. Postnatal evaluation by physical exam and/or karyotype is indicated after delivery.

How can I find a genetic counselor?

Genetic counselors are health care professionals with specialized training in cfDNA screening and the psychosocial complexities surrounding genetic testing and screening. They can help you explain these results to your patient, help your patient understand the genetic condition and facilitate a decision about further testing. A genetic counselor can be located using the “Find a Genetic Counselor” link on the nsgc.org website. You can also find more information about individual genetic conditions and national advocacy organizations for these conditions at www.lettercase.org/prenataltesting/.

References:

1. Wang JC, Sahoo T, Schonberg S, Kopita KA, Ross L, Patek K, Strom CM. Discordant noninvasive prenatal testing and cytogenetic results: a study of 109 consecutive cases. Genet Med. 2014 Aug 7.
2. Bianchi DW, Parker RL, Wentworth J, Madankumar R, Saffer C, Das AF, Craig JA, Chudova DI, Devers PL, Jones KW, Oliver K, Rava RP, Sehnert AJ; CARE Study Group. DNA sequencing versus standard prenatal aneuploidy screening. N Engl J Med. 2014 Feb 27; 370(9):799-808.
3. Choy KW, Kwok KY, Lau ET, et al. Discordant karyotype results among noninvasive prenatal screening positive cases. In: The Shifting Landscape of Genetic Testing: Approaches and Success Stories, Platform Session, Abstract #19, American Society of Human Genetics 2013 Annual Meeting, Boston, MA, USA, 2013.
4. Meck JM, Dugan EK, Aviram A, et al. Non-invasive prenatal screening: a cytogenetic perspective. In: Oral Platform Presentations: Cytogenetics, Abstract #17, American College of Medical Genetics and Genomics 2014 Annual Meeting, Nashville, Tennessee, USA, 2014.
5. Norton ME, Jacobsson B, Swamy GK, et al. Cell-free DNA analysis for noninvasive examination of trisomy. N Engl J Med. 2015 Apr 23; 372(17):1589-97.